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STANDARD OPERATING PROCEDURE

SOP Title: Improved Method for Extraction and Analysis of Perfluorinated Compounds (PFCs) from Surface Waters and Well Water by Ultra-High Performance Liquid Chromatography (UPLC)-Tandem Mass Spectrometry (MS/MS)

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Standard Operating Procedure for the Improved Method for Extraction and Analysis of Perfluorinated Compounds (PFCs) from Surface Waters and Well Water by Ultra-High Performance Liquid Chromatography (UPLC)-Tandem Mass Spectrometry (MS/MS)

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1. Scope and Applicability

This standard operating procedure (SOP) describes a method for the extraction, concentration, and analysis of perfluorinated compounds (PFCs) from surface waters and well water, using ultra-high performance liquid chromatography (UPLC)-tandem mass spectrometry (MS/MS). Details about how to operate the UPLC-MS/MS are not specified in this SOP.

2. Summary of Method

Oasis WAX solid phase extraction (SPE) cartridges are preconditioned for the collection and retention of PFCs. The target compounds are eluted with solvents and the resulting eluent is concentrated to 0.5 mL. The final concentrate is then diluted with buffer solution and analyzed by UPLC-MS/MS, operated in negative electro spray ionization (ESI) mode. Quantitation is completed using a multipoint calibration curve and internal standard calculation. The Limit of Quantitation (LOQ) of the method is 10.0 ng/L (10 ppt). Lower LOQs are achievable with this method, however for the developed calibration range

Note: For samples that are low volume or suspected to contain high concentrations a pre-screening may be performed prior to actual sample analysis. This step may aid in the determination of sample extraction volumes. See Section 10.2

3. Definitions

3.1 Sampling SOP

Sample collection, transportation, and storage are described in an independent SOP entitled "The Sample Collection Protocol for PFCs in Surface and Well Water" SOP # EMAB-113.0. This analytical SOP should be used as a companion to the Sample Collection SOP.

3.2 Internal Standard (IS)

A fixed amount of reference compound or solution is added to each sample and standard solution prior to extraction. The ratio of the detector signal of the native analyte to the detector signal of the internal standard (IS) is compared to the ratio obtained from the calibration curves where the IS level remains fixed and the native analyte levels vary. The internal standard is used to correct for minor sample-to-sample differences in extraction, purification, injection volume, chromatographic behavior, and mass spectrometry ionization efficiency. Internal standards used for this method are listed in Table 1b.

3.3 Quality Control (QC) Samples

Blanks, spikes, and duplicates are used as quality control measures.

Blanks are prepared with deionized laboratory water, and shipped to the field to assess potential contamination of sample containers, process reagents, and the analytical procedure.

For spikes, deionized water is spiked with known amounts of reference materials targeted by each analysis. The concentrations of analytes in these QC samples are compared to their theoretical values for the determination of the accuracy of the analysis.

Duplicate samples are sequential samples collected in the field to assess variability in the sample collection process.

4. Health and Safety

Personnel must be thoroughly acquainted with the potential hazards of the reagents, products, solvents, equipment, and procedures described in this SOP. The current Material Safety Data Sheets (MSDS) for the chemicals used in this method should be consulted. The Health and Safety Research Protocol for this laboratory activity is on file. Care should be observed with the use of all compounds specified in this protocol as some may be hazardous if used incorrectly (e.g., ammonium hydroxide, nitric acid).

5. Cautions

- **All labware and containers for samples and PFC solutions should be <u>polypropylene</u> to avoid sorption and loss of PFCs on the container walls.** Standard glassware may retain low levels of PFCs and may alter the precision and accuracy of the method.

- All labware should be washed with a non-phosphate alkaline soap solution, rinsed with warm water, followed by a final rinse with methanol or isopropanol prior to use to limit contamination and analyte carryover.

- Plastic-ware should be checked periodically for contamination, particularly when changing sources of supply or vendors. In the natural use of plastic-ware in the procedures, blank samples will show if contamination occurs.

- Strict QA/QC records for blanks and spiked samples should be maintained to assure continued quality assurance is met on a continual basis.

6. Interferences

Routine instrument maintenance is critical to achieve optimum method sensitivity. All laboratory materials must be determined to be free of contamination to ensure potential background interferences are minimized. This may be done by processing method blanks on a continuing basis in every assay. When contamination is noted the analysts should probe further to identify the source of contamination. UPLC column efficiency and chromatographic conditions must be maintained to ensure optimum separation of the target analytes. Note that increasing the sample volume loaded during SPE extraction will also increase the potential for matrix interference (see Section 10.4.3).

7. Personal Qualifications

This SOP assumes a thorough working knowledge of basic laboratory skills, reagents, and instrumentation. This document is designed to guide a competent laboratory worker in the analysis of PFC compounds and it is not intended to instruct individuals on the basic aspects of analytical chemistry.

8. Equipment and Supplies

8.1 Equipment

8.1.1 UPLC-MS/MS system

Waters Acquity (UPLC) ultra-high performance liquid chromatography system (Waters Corporation, Milford, MA) with binary pump, auto sampler, column heater, or other equivalent automated HPLC system suitable for the instrument parameters outlined in this method.

The UPLC used in this SOP was modified as follows:

1. All solvent lines were replaced with (polyether ether ketone) PEEK tubing
2. Mobile phase OEM PTFE filters were replaced with OEM stainless-steel filters.
3. Waters ACQUITY UPLC total solution for the analysis of Perfluorinated Compounds (PFCs) P/N 176001744 kit installed. Includes PFC isolator column and stainless steel tubing installed between the mixing chamber and injection port.
4. Original sample loop (10 µL) and injection syringe (10 µL) were replaced with 50 µL and 250 µL ones, respectively for increased sample injection volume capacity.

Waters Quattro Premier XE triple quadrupole mass spectrometer or other equivalent mass spectrometer that can achieve the instrument sensitivity outlined in this method.

Waters Acquity UPLC BEH C18 reverse phase UPLC column, 2.1×50 mm, 1.7 µm (P/N 186002350) or equivalent column suitable for resolution and retention of target PFC compounds.

8.1.2 TurboVap LV nitrogen evaporator (model # 103198, Caliper Life Sciences, Hopkinton, MA) or equivalent nitrogen evaporator/heated water bath capable of heating 25–60°C, with 10–15 psi nitrogen.

8.1.3 Dual piston syringe pump (CHRATEC SepPak Concentrator, Waters Corporation, SPC10-C) or equivalent system capable of variable flow rate and positive displacement.

Note: Vacuum manifold may be used in place of a syringe pump but sample concentration times and elution rates will be more variable, with less reproducibility. In addition, turbid samples even after filtering will clog SPE cartridges and prevent water passage dependent on vacuum.

8.1.4 Balance, electronic, capable of weighing to 0.1 mg (Mettler AB204S or equivalent)

8.1.5 General house vacuum for sample filtration, SPE loading, and/or elution.

8.2 Supplies

8.2.1 Glass Microfiber Filters, GF/A glass fiber filters (70 mm diameter) (Whatman, Florham Park, NJ, P/N 1820-070)

8.2.2 Two-piece polypropylene (70 mm) Buchner funnel (ACE Glass, P/N 12557-09 or equivalent

8.2.3 Vacuum Flask, liter size, polypropylene (Nalgene, 1000 mL) or equivalent.

8.2.4 SPE cartridge, Oasis WAX Plus extraction cartridge, 225 mg, 60 µm (Waters Corporation, P/N 186003519) or equivalent

8.2.5 Sample container, high density polyethylene HDPE, 1000 mL, wide mouth, with screw top (P/N 2189-0032 24/case Nalgene Labware, Rochester, NY) or equivalent

8.2.6 Centrifuge tube and cap, polypropylene, sterile, 15 mL (BD Falcon brand, BD, Franklin Lakes, NJ, P/N 352096, or equivalent)

8.2.7 Pipettor, variable volume, positive displacement, capability of 10 to 100, and 100 to 1000 microliter ranges (Eppendorf, Westbury, NY), or equivalent

8.2.8 Pipettor, repeating, positive displacement, capability of 1 to 50 mL (Eppendorf) or equivalent

8.2.9 Disposable polypropylene pipettor tips of various sizes (10, 100, 1000, 5000 uL) (Genesee Scientific, Research Triangle Park, North Carolina USA) or equivalent

*Note: Caution should be taken to avoid low binding pipette tips. Some vendors coat tips with coatings that contain polyfluorinated compounds that can add significant contamination. The user should assess pipette tip being used for contamination through the preparation of blank samples.

9. Reagents

9.1 Reference Materials

Reference materials including the perfluorinated analytes, the stable isotope internal standards, abbreviations, LC/MS/MS transitions, confirmation ions, and ion ratios monitored in analysis are listed in Table 1a and 1b below.

Target compounds were purchased in premixed ampoules prepared Wellington Laboratories, Guelph, Ontario, Canada (http://www.well-labs.com/). The PFAC-24PAR standard contains 24 perfluorinated analytes at 2 µg/mL (= 2 ng/µL) see Table 1a.

Internal standards were purchased as a mixture from Wellington Laboratories, Guelph, Ontario, Canada (http://www.well-labs.com/) (MPFAC-24ES) and include 19 stable isotope labeled perfluorinated analytes at 1 ug/mL (= 1 ng/uL) see Table 1b.

Additional internal standards do become available as time progresses. Ideally a matched internal standard to each analyte should be used. At the time this method was developed these were the existing commercially available internal standards.

Table 1a. Perfluorinated analytes, abbreviations, LC-MS/MS transitions, and confirmation ions.

Analyte	Abbreviation	Quant. transition	Confirm. transition
Perfluorobutanoic acid	PFBA	212.8 → 168.8	N/A
Perfluoropentanoic acid	PFPeA	262.9 → 218.8	N/A
Perfluorohexanoic acid	PFHxA	312.7 →268.7	312.7 →118.7
perfluoroheptanoic acid	PFHpA	362.7 → 318.7	362.7 → 168.7
perfluorooctanoic acid	PFOA	412.6 → 368.7	412.6 → 168.7
perfluorononanoic acid	PFNA	462.6 → 418.6	462.6 → 218.8
perfluorodecanoic acid	PFDA	512.6 → 468.6	512.6 → 468.6
perfluoroundecanoic acid	PFUdA	562.6 → 268.7	562.6 → 518.6
perfluorododecanoic acid	PFDoA	612.6 → 168.8	612.6 → 568.6
perfluorotridecanoic acid	PFTrDA	662.7 → 168.8	662.7 → 618.6
perfluorotetradecanoic acid	PFTeDA	712.7 → 318.8	712.7 → 668.7
perfluorobutane sulfonate	PFBS	298.7 → 79.9	298.7 → 98.8
perfluoropentane sulfonate	PFPeS	349.0 → 80.0	349.0 → 99.0
perfluorohexane sulfonate	PFHxS*	398.7 → 79.9	398.7 → 98.8
perfluoroheptane sulfonate	PFHpS	448.7 → 79.9	448.7 → 98.8
perfluorooctane sulfonate	PFOS*	498.7 → 79.9	498.7 → 98.8
perfluorononane sulfonate	PFNS	549.0 → 80.0	549.0 → 99.0
perfluorodecnane sulfonate	PFDS	598.6 → 79.9	598.6 → 98.8
perfluorooctane sulfonamide	PFOSA	498.0 → 77.9	498.0 → 168.9
N-methylperfluorooctane sulfonamidoacetic acid	N-MeFOSAA	570.0 → 419.0	NA
N-ethylperfluorooctane sulfonamidoacetic acid	N-EtFOSAA	584.0 → 419.0	NA
1H,1H,2H,2H-perfluorohexane sulfonate	4:2 FTS	327.0 → 81.0	327.0 → 307.0
1H,1H,2H,2H-perfluorooctane sulfonate	6:2 FTS	426.7 → 80.9	426.7 → 406.7
1H,1H,2H,2H-perfluorodecane sulfonate	8:2 FTS	526.8 → 80.8	526.8 → 506.7

* Analyte exists in the standard as the linear and branched isomers

Table 1b. Stable isotope labeled internal standards, abbreviations, LC-MS/MS transitions, and assigned analytes.

Isotope Labeled Standards	Abbreviation	Quant. transition	Assigned Analyte(s)
Perfluoro[$^{13}C_4$]butanoic acid	MPFBA	216.8 → 171.8	PFBA
Perfluoro[$^{13}C_5$]pentanoic acid	M5PFPeA	268.0 → 223.0	PFPeA
Perfluoro[$^{13}C_5$]hexanoic acid	M5PFHxA	318.0 →273.0	PFHxA
Perfluoro[$^{13}C_4$]heptanoic acid	M4PFHpA	367.0 → 322.0	PFHpA
Perfluoro[$^{13}C_8$]octanoic acid	M8PFOA	420.7 → 375.8	PFOA
Perfluoro[$^{13}C_9$]nonanoic acid	M9PFNA	472.0 → 427.0	PFNA
Perfluoro[$^{13}C_6$]decanoic acid	M6PFDA	519.0 → 474.0	PFDA
Perfluoro[$^{13}C_7$]undecanoic acid	M7PFUdA	570.0 → 525.0	PFUdA
Perfluoro[$^{13}C_2$]dodecanoic acid	MPFDoA	615.0 → 570.0	PFDoA, PFTrDA
Perfluoro[$^{13}C_4$]tetradecanoic acid	M2PFTeDA	715.0 → 670.0	PFTeDA
Perfluoro[$^{13}C_3$]butane sulfonate	M3PFBS	301.7 → 79.8	PFBS, PFPeS
Perfluoro[$^{13}C_3$]hexane sulfonate	M3PFHxS	402.0 → 99.0	PFHxS, PFHpS
Perfluoro[$^{13}C_8$]octane sulfonate	M8PFOS	507.0 → 99.9	PFOS, PFNS, PFDS
Perfluoro[$^{13}C_8$]octane sulfonamide	M8PFOSA	506.0 → 78.0	PFOSA
N-[d_3]methylperfluorooctane sulfonamidoacetic acid	d3-N-MeFOSAA	573.0 → 419.0	N-MeFOSAA
N-[d_5]ethylperfluorooctane sulfonamidoacetic acid	d5-N-EtFOSAA	589.0 → 419.0	N-EtFOSAA
1H,1H,2H,2H-perfluoro[1,2-$^{13}C_2$]hexane sulfonate	M2-4:2 FTS	329.0 → 81.0	4:2 FTS
1H,1H,2H,2H-perfluoro[1,2-$^{13}C_2$]octane sulfonate	M2-6:2 FTS	428.7 → 408.7	6:2 FTS
1H,1H,2H,2H-perfluoro[1,2-$^{13}C_2$]decane sulfonate	M2-8:2 FTS	529.0 → 81.0	8:2 FTS

9.2 Chemicals/Solvents

9.2.1 Methanol (MEOH), HPLC grade or equivalent (Honeywell Burdick & Jackson, Muskegon, MI)

9.2.2 Water, HPLC grade or deionized (DI water), predetermined to be PFC free based on analysis using this SOP

9.2.3 Ammonium Acetate, Fluka, puriss. p.a. grade, for mass spectroscopy, ≥ 99.0% (calc. on dry basis) (P/N 17836) or equivalent

Caution: Reagent grade ammonium acetate is hydroscopic—protect from water adsorption, maintaining in desiccator when not in use.

9.2.4 Glacial acetic acid, Sigma-Aldrich (St. Louis, MO)

9.2.5 Sodium acetate, Sigma-Aldrich (St. Louis, MO)

9.2.6 Ammonium hydroxide (NH_4OH, 28% in water), Sigma-Aldrich (St. Louis, MO)

9.2.7 Five mL ampoules of 35% nitric acid, EP Scientific Products – Thermo Fisher Scientific (P/N SVCN-5-1). *Alternatively, 5 mL ampules can be made in-house as a 50:50 mix of house DI water:concentrated nitric acid. However, assessment of blanks samples due to in-house preparation should be determined prior to use in field sampling efforts.

10. Procedures for Solution Preparation and Sample Extraction

10.1 Preparation of Solutions

10.1.1 Ammonium Acetate Buffer Solution Preparation

***Stock Solution*:** Prepare a 100 mM solution of ammonium acetate by weighing 7.7 g (100% purity, anhydrous basis) per liter of deionized or HPLC grade water in a suitable storage container. Cap and shake well by hand inversion until clear and all crystals are dissolved. Label and refrigerate 4°C for long term storage.

***Working solution*:** Prepare as needed a 2 mM solution of ammonium acetate by warming the stock solution to ambient temperature and pipetting a 20 mL aliquot of the stock solution and diluting to 1 L volume with deionized or HPLC grade water. Make sure no precipitate is formed.

10.1.2 Internal Standard Preparation

***Stock Solution*:** The internal standard (IS) should be prepared so that the IS level for each compound approximates the midpoint on the standard curve. It must also be accurately and precisely delivered to each unknown, QC, and calibration sample used in the analysis.

All IS materials are supplied from the manufacturer as a mixture in methanol/isopropanol/water solutions at 1 ng/µL.

Each 1 liter of unknown, QC, or calibration sample prepared will therefore receive 50 µL IS stock solution, delivering 50 µL x 1 ng/µL = 50 ng/L of each of the IS materials.

10.1.3 Calibration Solution Preparation

The standard curve covers all of the target compounds included in the PFAC-24PAR standard purchased from Wellington Laboratories, Guelph, Ontario, Canada (http://www.well-labs.com/). Table 1a indicates the analytes included in this mixed standard.

A 6-8 point calibration curve should be prepared to bracket the range of concentrations expected in the samples being analyzed. This range can be adjusted for targeting higher or lower concentration ranges.

Refer to Table 2 below for an example calibration standards preparation.

Table 2. Example preparation of calibration standards.

Calibration Point (ng/L)	Volume DI water (mL)	Volume standard solution (uL)	Concentration in standard solution (ng/uL)	Total mass of each analyte added (ng)
10	1000	5	2.0	10
25	1000	12.5	2.0	25
50	1000	25	2.0	50
75	1000	37.5	2.0	75
100	1000	50	2.0	100
125	1000	67.5	2.0	125
150	1000	75	2.0	150
200	1000	100	2.0	200

Each of these calibration samples is prepared as indicated above, receiving the appropriate standard solution, the IS solution, and 5 mL of 35% nitric acid, used as a preservative for the unknown and QC samples.

10.1.4 QC Spike Preparation

Field spikes can be prepared at low (ex. 25 or 50 ng/L) and high (ex. 50 or 100 ng/L) levels of all of the compounds on the target list. These concentrations were chosen to bracket health advisory values for PFOS and PFOA respectively. More appropriate or additional spike levels can be prepared and assessed if needed. These QC samples will be prepared in the laboratory and shipped with the empty sample containers to be used for the collection surface or well water samples. Preparation will be as follows:

PFAC-24PAR standard containing all of the target compounds at 2 µg/mL (= 5 ng/µL) will be purchased from Wellington Laboratories (http://www.well-labs.com/).

For example, to prepare a low level field spikes at 50 ng/L, 25 µL of this standard will be added to 1 L of deionized laboratory grade water (2 ng/µL x 25 µL = 50 ng/L) and capped and mixed well. The samples will be preserved with the addition of 5 mL of 35% nitric acid, supplied in premeasured ampoules. These low level field spikes will be prepared at a rate of 10% of all unknown water samples.

To prepare high level field spikes, 50 µL of this standard will be added to 1 L of deionized laboratory grade water (2 ng/µL x 50 µL = 100 ng/L) and capped and mixed well. The samples will be preserved with the addition of 5 mL of 35% nitric acid, supplied in premeasured ampoules. These high level field spikes will be prepared at a rate of 10% of all unknown water samples.

Additional low and high trip spikes may be prepared for a lower or higher range calibration curve range.

Note: Different vials or lots of the native standards should be used to prepare the QC spikes and the calibration samples if available.

10.1.5 Sodium Acetate Buffer Preparation (pH 4)

- Used for SPE conditioning and washing

Dilute 1.4 mL of glacial acetic acid with DI water in a glass bottle to make 1-L of 25 mM acetic acid. Weigh out 3.4 g of sodium acetate and dissolve in DI water in a glass bottle bringing up the final volume to 1 L. Mix 800 mL of acetic acid solution and 200 mL of sodium acetate solution to make the 25 mM sodium acetate buffer (pH 4).

10.1.6 Methanol/Ammonia Solution Preparation

- Used for sample elution from SPE cartridge (see Section 10.4.5)
- Refer to Table 3 for elution solvent preparation.

On every day of the analysis, a fresh mixture of this solution should be made to adequately cover the volume needed for the experiment. For example, 10 mL of solution will be required for each sample (5 mL to condition each SPE cartridge and 5 mL for analyte elution). If 100 mL of solution is to be made, mix 100 uL of ammonium hydroxide (28% in water) with 100 mL of methanol and shake vigorously. Although this solution is good for more than one day, it is best to make this solution up fresh daily since ammonia is volatile.

Table 3. Volumes for the preparation of elution solvent.

Volume of Ammonium Hydroxide (28%) (uL)	Volume Methanol (mL)	Desired volume of elution solvent (mL)	Maximum number of WAX cartridges
100	100	100	10
200	200	200	20
300	300	300	30
400	400	400	40
500	500	500	50

10.2 Sample Pre-screening (Optional)

For samples that are limited in volume or are suspected of having high concentrations that may exceed calibration levels, analytical pre-screening may be performed to guide in sample extraction volumes. Samples may be pre-screened by taking 300 uL of each water sample and combining with 100 uL of methanol into an HPLC vial followed by vortex mixing (10 seconds) and UPLC-MS/MS analysis. Appropriate calibration curve points may be constructed by adding analytes of interest to DI water, vortex mixing (10 seconds) and sampling as above (300 uL water:100 uL methanol in HPLC vial). For example, a 7-point calibration curve may include points from 200 ng/L up to 3000 ng/L. Internal standards should be added at a concentration approximating the midpoint of the calibration curve.

10.3 Sample Pretreatment and Filtration

10.3.1 Volume Measurement

Pour whole water sample into a previously cleaned 1-L HDPE graduated cylinder and record the volume.

10.3.2 Methanol Rinse of the Sample Container

Add 10 mL of methanol into the emptied bottle, cap, and shake well. **Do not discard the rinse.** Pour the sample that is in the graduated cylinder back into the original sample bottle and mix with the methanol rinse.

Note: This step must be performed immediately prior to the filtration steps that follow (in Section10.3.4).

10.3.3 Internal Standard Addition

Add appropriate volume of working internal standard solution (containing enough mass of the IS to approximate the midpoint of the standard curve) to each sample and mix well.

10.3.4 Filtration

Filter the whole sample with GF/A glass fiber filter under gentle vacuum into a previously cleaned 1-liter vacuum flask. If the filter apparatus clogs during filtration due to a highly turbid sample, a new filter can be exchanged for the clogged one and filtration completed. This should be noted in one's notebook.

If any particulate matter remains in the original sample bottle, rinse it clean by adding 20 mL of DI water, mixing thoroughly, and discarding this rinsate to waste. After rinsing the bottle, pour the entire filtered sample back into the original bottle for the sample extraction process.

10.4 Sample Extraction

The following procedure is a general method for the capture and elution of PFCs using a dual piston pump system for the concentration of analytes on an Oasis WAX solid phase extraction (SPE) cartridge. Alternate means of collection and concentration can be used but are not addressed in this SOP.

10.4.1 Conditioning of Cartridge

Pass methanol through each SPE cartridge using the SPE pumps at a rate of 10 mL/min for 2.5 minutes. Alternatively, SPE cartridges may be conditioned by passing 5 mL of methanol through the cartridges with a vacuum manifold. Discard all solvents to waste.

10.4.2 Equilibration of Cartridge

Using a dual piston pump system, run 25 mL of DI water through the cartridge at a flow rate of 10 mL/min for 2.5 minutes to remove the methanol. Discard the water to waste. (The flow rate for all procedures using the dual piston pump is 10 mL/min).

10.4.3 Loading Samples onto Cartridge

Pass 500 mL of sample water containing the IS through the cartridge at a steady flow rate of 10 mL/min using the dual piston pump. Water that passes is discarded to waste.

Note: If more sensitivity is required, the volume of sample loaded onto the SPE cartridge can be increased. However, the sample volume must be known and recorded for later calculation of the concentration (wt/volume). It is also important to note that increasing the sample volume will also increase the potential for matrix interference.

10.4.4 Cartridge Wash

Place the cartridge on a vacuum manifold and apply vacuum long enough to remove any obvious water drops remaining in the cartridge. Pass 4 mL of 25 mM sodium acetate buffer (pH 4.0) and discard to waste.

10.4.5 Analyte Elution and Collection

Fit the corresponding SPE cartridge on the top of the manifold to prepare for sample elution. Wash the SPE cartridge with 4 mL of 100% methanol and discard to waste except for samples needing PFOSA analysis*.

***PFOSA Collection**: For the collection of PFOSA, place a 15 mL polypropylene centrifuge tube in the collection reservoir of the vacuum manifold to capture the 4 mL of 100% methanol passed through the SPE cartridge using the WAX method that is otherwise discarded to waste. As PFOSA is not anionic, it is not retained at this step and will be lost unless captured. Remove the tube from the rack, vortex mix, and then evaporate the eluate down to approximately 500 to 1000 µL (at 40°C) with TurboVap LV or similar nitrogen/bath evaporator. Do NOT completely evaporate the eluate.

Collection of All Other Analytes: After the methanol wash, place a 15 mL polypropylene centrifuge tube in the collection reservoir of the vacuum manifold to collect the final eluate. Run 4 mL of methanol/ammonia solution through the cartridge at a flow rate of approximately 1 drip/second. Remove the tube from the rack, vortex mix, and then evaporate the eluate down to approximately 500 to 1000 µL (at 40°C) with TurboVap LV or similar nitrogen/bath evaporator.

Note: Eluate should NOT be completely evaporated, which may cause loss of analytes. A lower temperature is recommended because shorter chain species may evaporate off at higher temperatures.

10.4.6 Preparation for Instrumental Analysis

Dispense 300 µL of 2 mM ammonium acetate buffer into all HPLC auto sampler vials. Transfer 100 µL of each eluate into a respective auto sampler vial such that the final solution is 75% buffer and 25% methanol by volume.

Note: If specific UPLC conditions need to be changed to provide optimal peak shape and separation of compounds, then modify the buffer/methanol mobile phase ratio to match the initial UPLC flow conditions.

Cap and vortex each vial well for more than 10 seconds to ensure solution uniformity. Analyze the samples with UPLC-MS/MS using the conditions outlined in Section 11.

11. Determination of Analyte Concentration

11.1 UPLC

Using the instrument system and column outlined in Section 8.1.1, set up the method conditions using the following parameters:

Reservoir **A**: 2 mM ammonium acetate in DI water with 5% methanol
B: 2 mM ammonium acetate in 100% methanol
Column: BEH C18 reverse phase, 2.1×50 mm, 1.7 µm particle size
Flow rate: 500 µL/min
Column temperature: 50°C
Injection Volume: 50 µL
Gradient mobile phase program:

Time (min.)	A	B	Curve
0.00	75	25	initial
0.50	75	25	6
5.0	15	85	6
5.1	0	100	6
5.6	0	100	6
7.0	0	100	1
9.0	75	25	1
12.0	75	25	initial

Note: The gradient program should be adjusted, if necessary, for the complete separation of all analytes from any matrix interferences.

11.2 MS/MS

The Quattro Premier mass spectrometer is operated in the multiple reaction monitoring (MRM) mode using negative-ion-spray ionization under the following conditions:

Instrument Parameters	
Capillary (kV)	−0.40
Source temperature	150°C
Desolvation temperature	350°C
Cone gas flow	2 L/hr
Desolvation gas flow	1200 L/hr
Cone voltage	Optimized for
Collision energy	each compound

Note: Ionization and collision cell parameters are optimized for each individual analyte (See Section 13).

11.3 System Conditioning

From 1-3 double blanks containing unprocessed methanol and 2 mM ammonium acetate, (in a 1:3 ratio) should be injected as the first part of any sample batch in order to ensure that minimal background signal is achieved. Henceforth, an additional double blank should be inserted every 10th sample to ensure system background performance. PFCs are common contaminants in most analytical systems, but as long as any peak areas in the double blanks are at least 10 times smaller than peak areas in the lowest point on the calibration curve, adequate sensitivity has been achieved.

If an isolation column is used (see Section 8.1.1) to separate background systemic PFAS contamination from sample contamination, three double blanks should be sufficient to achieve starting chromatographic conditions. Separation of an analytical peak (a peak due to sample injection) and a background peak (systemic contamination from solvents, internal components) should achieve baseline resolution. The earlier eluting peak is the analytical peak whereas the later eluting peak is systemic. It is not uncommon for a system that has sat dormant for some time to take many injections until systemic contamination is low and consistent. However, it is not common for analytical peaks in blank samples to remain high and consistent. If this occurs it is likely that contamination from laboratory apparatus or solvents is the cause (i.e. HPLC vials and caps, solvents, pipette tips). If this occurs the source of the contamination should be identified and corrected.

11.4 Integration

Peak area counts for each analyte and internal standard are automatically determined using the mass spectrometer software (MassLynx 4.1). Each chromatogram should be reviewed by the operator to insure proper and consistent integration, and manual corrections of inappropriate integrations or missed peaks should be applied if necessary.

12. Calculation

Calculations are based on the isotope dilution method of quantitation. Calculations may also be performed by automated data system, i.e. MassLynx or other equivalent software.

A calibration curve is created separately from the analysis of each standard solution for each analyte. The peak area count ratio of the analyte to the internal standard (y-axis) versus the concentration of analyte (x-axis) is plotted and a best fit regression formula is determined using $1/x$ weighting. The resulting standard curves are used for calculation of analyte concentration in the unknown samples. Each standard curve should result in a coefficient of determination (r^2) of greater than 0.99 for a seven point curve.

Note: If samples are found to exceed the top of the calibration curve, they will need to be diluted and reanalyzed. Using and estimation of the concentration in the sample, dilute the sample(s) to approximate the mid-point of the calibration curve. Samples should be diluted using DI water with nitric acid added containing IS/liter of water at the same concentration as in previously prepared samples. Calculation of analyte concentrations must include correction for the dilution factors used to prepare the sample.

13. Specification Parameters

The following instrumental parameters were determined to be adequate in repeated experiments involving quantitation of PFCs in surface water. Different instruments will have their own unique settings that will need to be optimized depending on each laboratory's specific operating conditions. An example of these conditions is shown in Table 4.

Table 4. Specific parameters for Quattro Premier XE (MS/MS) broken into 3 sequential time segments. Primary transitions are indicated by °1 and confirmation transitions by °2 designations.

Time segment 1 (0-3.5 minutes)

Analyte	Parent Ion	Daughter Ion	Cone Voltage	Collision Energy
PFBA	212.8	168.8	15	10
PFBA IS	216.8	171.8	15	10
PFPeA	262.9	218.8	15	9
PFPeA IS	268.0	223.0	15	9
PFBS °1	298.7	79.9	40	30
PFBS °2	298.7	98.8	40	28
13C3-PFBS	301.7	79.8	60	30
PFHxA °1	312.7	118.7	13	21
PFHxA °2	312.7	268.7	13	10
PFHxA IS	318.0	273.0	13	9
4:2 FTS °1	327.0	81.0	43	30
4:2 FTS°2	327.0	307.0	43	20
4:2 FTS IS	329.0	81.0	43	30
PFPeS °1	349.0	80.0	45	34
PFPeS °2	349.0	99.0	45	30

Time Segment 2 (3.5 – 5 minutes)

Analyte	Parent Ion	Daughter Ion	Cone Voltage	Collision Energy
PFHpA °1	362.7	168.7	14	17
PFHpA °2	362.7	318.7	14	10
PFHpA IS	367.0	322.0	14	10
PFHxS °1	398.7	79.9	50	38
PFHxS °2	398.7	98.8	50	32
PFHxS IS	402.0	99.0	50	38
PFOA °1	412.6	168.7	15	18
PFOA °2	412.6	368.7	15	11
13C8 PFOA IS	420.7	375.8	15	11
6:2 FTS °1	426.7	80.9	43	34
6:2 FTS °2	426.7	406.7	43	24
6:2 FTS-IS	428.7	408.7	43	24
PFHpS °1	448.7	79.9	60	48
PFHpS °2	448.7	98.8	60	38
PFNA °1	462.6	218.8	15	17
PFNA °2	462.6	418.6	15	11

Time Segment 2 (3.5 – 5 minutes) continued				
Analyte	**Parent Ion**	**Daughter Ion**	**Cone Voltage**	**Collision Energy**
PFNA IS	472.0	427.0	15	11
PFOS °1	498.7	79.9	60	48
PFOS °2	498.7	98.8	60	38
13C8 PFOS IS	507.0	99.0	60	48

Time Segment 3 (5-12 minutes)

Analyte	**Parent Ion**	**Daughter Ion**	**Cone Voltage**	**Collision Energy**
PFOSA °1	498.0	77.9	50	32
PFOSA °2	498.0	168.9	50	28
PFOSA IS	506.0	78.0	50	32
PFDA °1	512.6	218.8	16	18
PFDA °2	512.6	468.6	16	12
PFDA IS	519.0	474.0	16	12
8:2 FTS °1	526.8	80.8	45	45
8:2 FTS °2	526.8	506.7	45	28
8:2 FTS IS	529.0	81.0	45	45
PFNS°1	549.0	80.0	65	51
PFNS°2	549.0	99.0	65	41
PFUnA °1	562.6	268.7	18	19
PFUnA °2	562.6	518.6	18	12
MeFOSAA	570.0	419.0	35	20
PFUnA IS	570.0	525.0	17	12
MeFOSAA IS	573.0	419.0	35	20
EtFOSAA	584.0	419.0	35	20
EtFOSAA IS	589.0	419.0	35	20
PFDS °1	598.6	79.9	73	55
PFDS °2	598.6	98.8	73	44
PFDoA °1	612.6	168.8	18	28
PFDoA °2	612.6	568.6	18	13
PFDoA IS	615.0	570.0	18	13
PFTrA °1	662.7	168.8	18	28
PFTrA °2	662.7	618.6	18	13
PFTA °1	712.7	318.8	19	22
PFTA °2	712.7	668.7	19	14
PFTA IS	715.0	670.0	19	14

14. Data and Records Management

Raw data (including electronic data on individual PC hard drives and group shared drives) should be backed up, maintained and made available for review. A consistent file naming convention should be documented and used for each specific project and data type generated. data generated should be maintained by the principal investigator until completion of the project. Upon completion, data should be stored in accordance with EPA's record management policy. All instrument data should be backed up to network drives on a regular basis and should be archived along with other supporting data and relative correspondence at the completion of the study. Printed data should be referenced, signed and dated in accordance with the Office of Research & Development's Policies and Procedures Manual

Section 13.02 on Paper Laboratory Records. The research notebook will be the record for any procedure conducted in the field or laboratory and will provide the objective, procedural details, data references and discussion for project development. These entries will give a full and complete statement of the situation being examined, a specific hypothesis relating to the situation, and a brief experimental protocol designed to answer the questions posed in the hypothesis. Data will be recorded from these experiments and a discussion of the results will be presented with conclusions drawn. Any standard, solution, or sample made during these investigations should be marked with a reference number, traceable to a specific entry in the research notebook. An example could be the initials of the analyst-lab notebook number-page number-sample number (ex. MJS 1-50-1). The laboratory notebooks are the property of EPA and will be stored in accordance with EPA's record management policy.

15. Quality Control and Quality Assurance

Analysis of standard solutions should result in a best fit regression coefficient of determination (r^2) of 0.99 or greater, using a minimum of seven independent concentrations that bracket the sample concentrations. Sample concentrations resulting in peak area ratios that are lower or greater than the range of standards should be reanalyzed with appropriate re-extraction or dilution of original water sample, respectively

Trip Spike Samples: For quality control checks, samples of deionized water are acidified with nitric acid and fortified at two concentrations that bracket the expected unknown sample concentrations of PFCs. These samples are unopened in the field, shipped with unknown samples and analyzed with each batch of water extracts. Based on the demonstrated precision data, the analyses are considered acceptable if the calculated concentrations are ±20% of the expected values.

Trip Blank Sample: One laboratory method blank consisting of 1 liter of DI water acidified with nitric acid and unfortified is analyzed for each batch of samples analyzed. This sample is unopened in the field, shipped with unknown samples and analyzed with each batch of water extracts. If significant analyte levels (S/N > 5) are found in the laboratory method blank, the source of contamination should be identified, corrected, and verified as being eliminated before additional analyses of unknown samples proceed.

For each field sampling event, it is recommended that 10% fortified DI water should be sent to the field along with blank sample containers. Duplicate samples should be collected from at least 10% of the sampling sites. Refer to Sampling SOP (EMAB-113.0) for the detail.

16. Method Validation

This method has been validated for the following parameters using natural water samples and deionized water. The results of this validation procedure are presented below, and typical chromatograms can be found in Appendix I. Previous calibration ranges and QA/QC and results are shown here for a subset suite of the total analyte list. Additional validation results are to follow with an appendix addition.

16.1 Specificity

Chromatographic examinations of method blanks, control samples (deionized water), and various natural water samples did not reveal any significant matrix interferences with each analyte or internal standard peak area.

16.2 Linearity

Each analyte solution was evaluated, in duplicate, and dilutions covered the range of 10 ng/L to 1000 ng/L. Each standard curve resulted in a coefficient of determination (r^2) of greater than 0.99 for a six point curve. Some analytes may have a better fit with a quadratic regression.

16.3 Precision

A bulk river sample was collected and split into six 500 mL subsamples. Each subsample was then prepared for analysis, representing six individual replicate analyses. The precision for all analytes based on relative standard deviation (RSD) resulted in < ± 20%.

16.4 Accuracy

Deionized water samples were spiked at either 200 ng/L (low QC) or 400 ng/L (high QC) for use as QC samples. Duplicate solutions were prepared for each daily analysis. The accuracy for all PFC analytes for QC samples both at 200 and 400 ng/L was in a range of 80 to 120% of the spiked concentration.

16.5 Recovery

Recoveries were calculated based on a matrix matched spike extraction. Pond water was spiked with known amounts of PFCs and then extracted using an SPE cartridge. After elution of the PFCs from the cartridge, the internal standards were added to the eluate. The same volume of un-spiked pond water was extracted using a second SPE cartridge. After elution, the same amount of PFCs and internal standards were added to the eluate. The area ratio of the first sample (pre-addition to cartridge) was divided by that of the second eluate (post-addition) to give a recovery determination. The equation used for the calculation is shown below. Each level had five replicates. The recoveries for each compound are illustrated in Figure 1 of Section 16.6.

$$Rc = \frac{SB - BLK}{SA - BLK}$$

Where Rc = recovery,
SB = response for SPE extract of matrix spiked before SPE
SA = response for SPE extract of matrix spiked after SPE
BLK = response for SPE extract of matrix without spike

16.6 Matrix Effect

Matrix effects were calculated comparing a blank matrix extract spiked with PFCs to a blank methanol solution spiked with the same amount of PFCs. Un-spiked pond water samples were extracted using the SPE method described above. After elution, a specific amount of PFCs and internal standards were added to the eluate. The same volume of methanol was spiked with the same amount of PFCs and internal standards. After subtracting the background level of PFCs found in the pond water, the area ratio of the first sample (post-elution spike) was divided by that of the methanol solution (solvent) to give a matrix effect determination. The equation used for the calculation is shown below. Each level had five replicates. The matrix effects for each compound are illustrated in Figure 1 below.

$$ME = \frac{SA - BLK}{Sstd}$$

Where ME = matrix effect (positive: ionization enhancement, negative: ionization suppression),

SA = response for SPE extract of matrix spiked after SPE
$Sstd$ = elution solvent spiked (no matrix)
BLK = response for SPE extract of matrix without spike

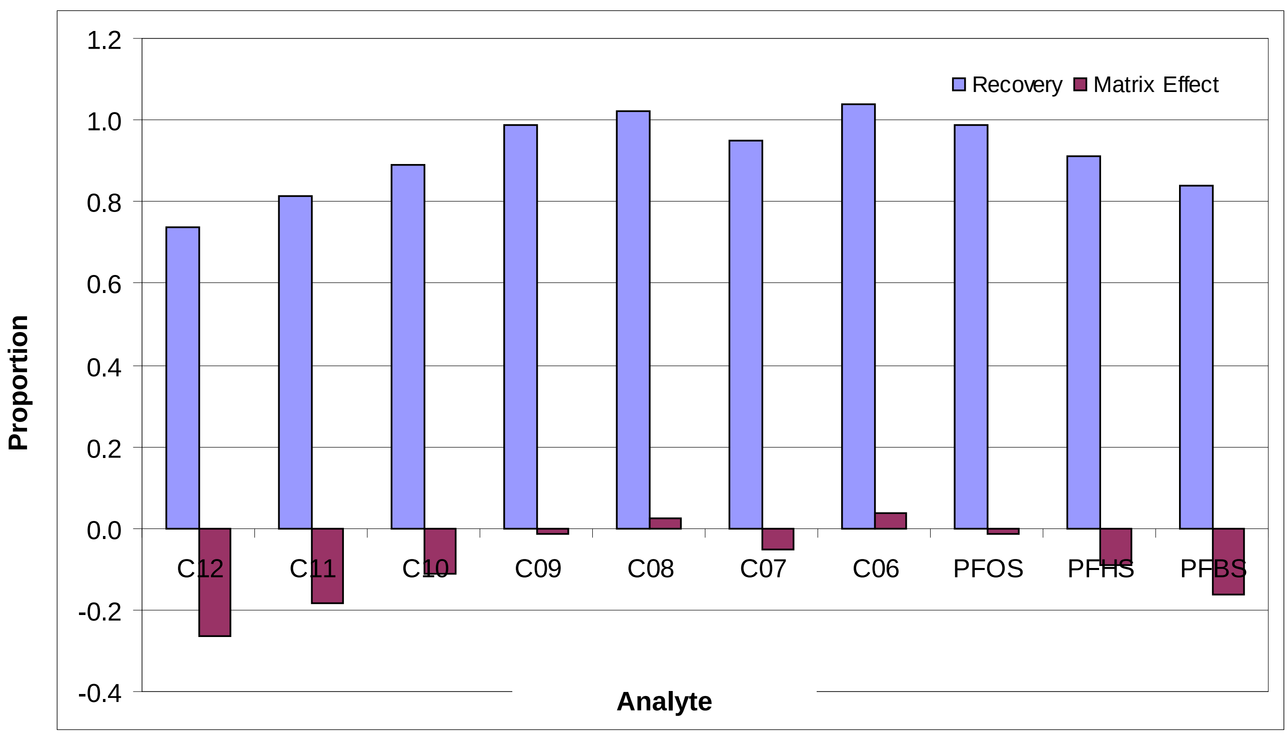


Figure 1. Recoveries and matrix effect for each compound.

16.7 Stability

16.7.1 Sample Stability

Samples are stable for a minimum of 4 weeks at ambient temperature if they are appropriately preserved (refer to the Sampling SOP (EMAB 113.0).

Note: Methanolic rinse of the sample container is essential in order to recover PFCs from sample container wall for the tested substances listed in this SOP. Additional compounds not listed in this SOP will need to be assessed for recovery and sample stability.

16.7.2 Cartridge Stability

The stability of PFCs from water samples that have been processed and captured on the WAX SPE cartridge was evaluated by storing sample loaded cartridges for one month at ambient temperature. No reduction in recovery was observed when compared to duplicate samples which were analyzed immediately after preparation.

16.7.3 Solution Stability

PFC standards and spiking solutions are stable for at least 2 months when stored at ambient temperature. Methanolic standards from the suggested vendor (Wellington Laboratories) come with NaOH added to prevent the formation of methyl esters of the analytes of interest. This leads to loss of analyte with time. This appears to only effect the perfluorinated carboxylic acids and not the sulfonic acids.

16.8 Limit of Detection

The limit of detection (LOD) and limit of quantitation (LOQ) for each PFC were determined by analyzing low concentration standards (~1/10 x the lowest calibration point) 6 times on 6 separate days. The LOD was calculated as follows;

$$\mathrm{LOD} = \frac{3 \times s}{m}$$

where *s* is the standard deviation of response on the MS/MS and *m* is the slope of the calibration curve. The LOD for this procedure has been determined to be 0.05 ng/mL for each analyte shown in Figure 1.

16.9 Limit of Quantitation

Using the procedure described above in Section 16.8 the limit of quantitation (LOQ) was determined as follows:

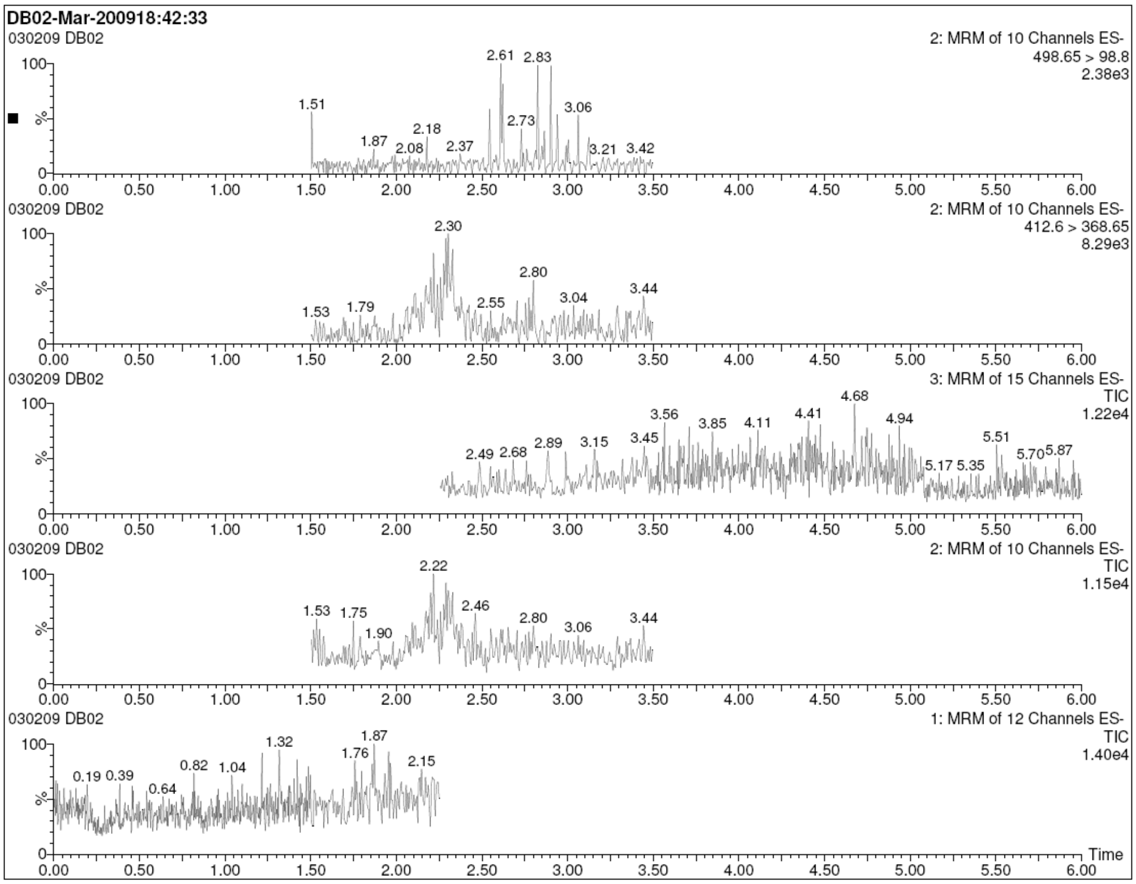
$$\text{LOQ} = \frac{10 \times s}{m}$$

The LOQ for this procedure has been determined to be 0.5 ng/mL for each analyte shown in Figure 1.

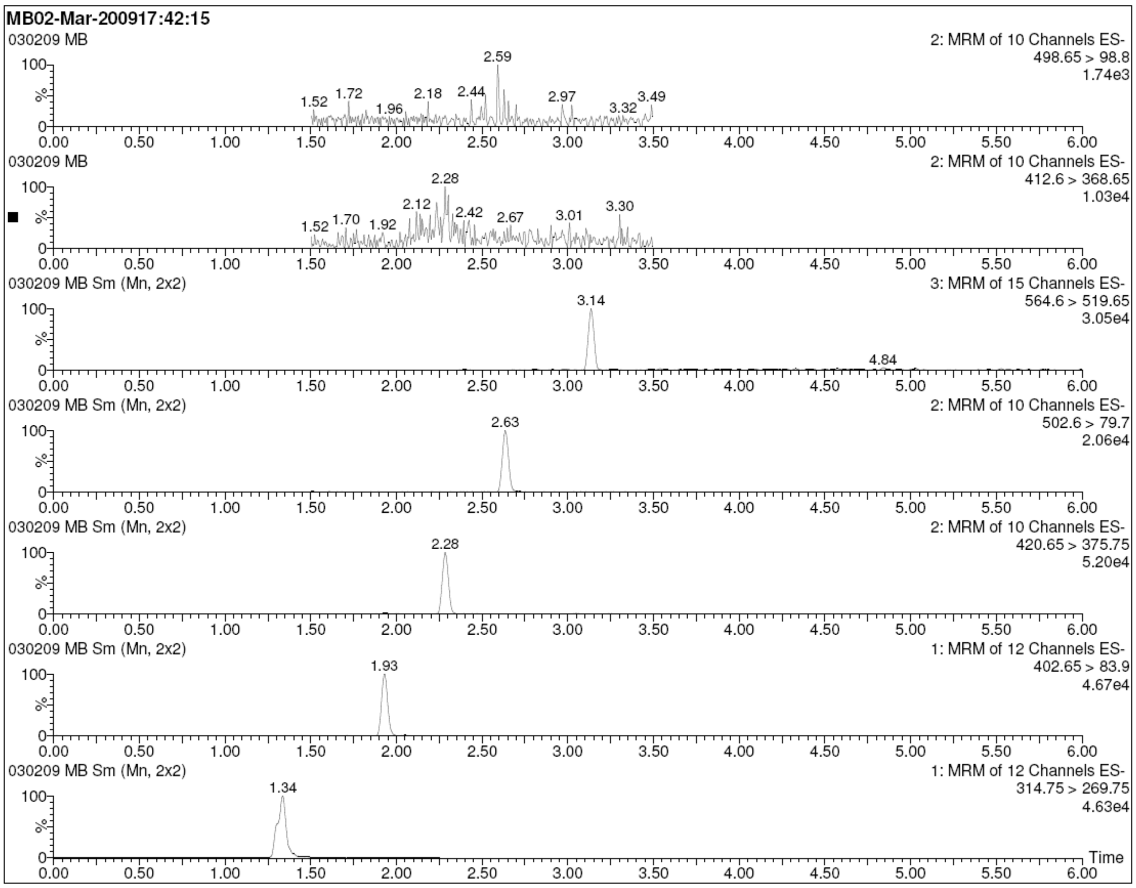
These procedures are outlined in Long G.L. and Winefordner J.D. Limit of detection. A closer look at the IUPAC definition. Analytical Chemistry, 1983, 55 (7), 712A-724A.

Appendix I. Typical Chromatograms

Example Solvent blank. Bottom 3 plots are total ion chromatograms (TIC) for all compounds and ISs. Top two plots are extracted ion chromatogram (EIC) for PFOA (412.65>368.65) and PFOS (498.65>98.8) primary MRMs, respectively.



Example Method Blank. Bottom 5 plots are EIC for C6-IS, PFHS-IS, PFOA-IS, PFOS-IS and C11-IS respectively. Top 2 plots are EIC for primary MRM for PFOA and PFOS, respectively.



Example Calibration Sample. Plots are of primary MRMs for each analyte starting from the bottom as PFBS, C6, C7, PFHS, C8, C9, PFOS, and C10, respectively.